

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
15 February 2001 (15.02.2001)

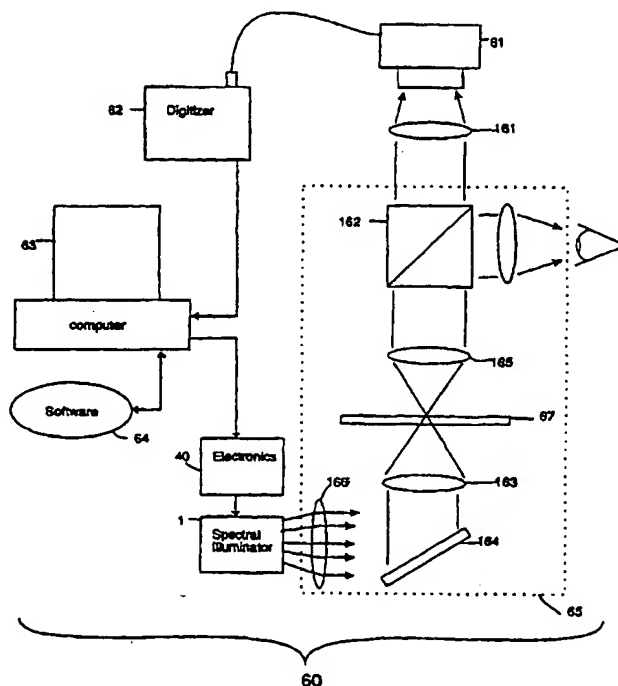
PCT

(10) International Publication Number
WO 01/11343 A1

- (51) International Patent Classification⁷: G01N 21/64, G01J 5/02
- (21) International Application Number: PCT/US00/21481
- (22) International Filing Date: 7 August 2000 (07.08.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 60/147,636 6 August 1999 (06.08.1999) US
- (71) Applicant: CAMBRIDGE RESEARCH & INSTRUMENTATION INC. [US/US]; 80 Ashford Street, Boston, MA 02134 (US).
- (72) Inventors: MILLER, Peter, J.; 146 High Street, Newburyport, MA 01950 (US). HOYT, Clifford, C.; 18 Newbury Park, Needham, MA 02192 (US).
- (74) Agent: PAVANE, Martin, B.; Cohen, Pontani, Lieberman & Pavane, 551 Fifth Avenue, Suite 1210, New York, NY 10176 (US).
- (84) Designated States (regional): European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
- Published:
— With international search report.
— Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.

[Continued on next page]

(54) Title: SPECTRAL IMAGING SYSTEM



(57) Abstract: An imaging system is disclosed comprising an illuminator (1) which produces illumination of any desired pure wavelength or of an selected mixture of pure wavelengths simultaneously, which illuminates a sample without spatio-spectral artifacts using illumination optics designed for that purpose; imaging optics (11a-11j), which form

[Continued on next page]

WO 01/11343 A1



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

an image of the sample at a detector or viewing port; and a detector. This enables imaging the complete spectral image cube for a sample by taking sequential images while illuminating with a series of pure wavelengths, with greater ease and economy than by means of tunable filters (12a-12j), interferometers and the like. It further enables imaging while the sample is illuminated with a precisely controlled mixture of illuminant wavelengths, so that the image presented to the detector is a linear superposition of the sample properties at many wavelengths.

Spectral Imaging System

RELATED APPLICATIONS

This application claims priority from U.S.
5 Provisional Patent Application No. 60/147,636, the
contents of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

In this application, a spectral weighting function
10 that indicates the presence or amount of a certain trait
in a sample is termed a spectral index, spectral
weighted index or spectral measure for that trait. The
term spectral measure is also sometimes used to denote
the measurement value obtained at a given point or
15 region according to a given spectral weighting function.

Various spectral imaging systems are used to derive
spectral information about samples, including imaging
spectrometers, imaging interferometers, band-sequential
cameras using e.g. filter wheels, and linear-variable
20 filter imagers. Those systems which are based on linear-
variable filters or on spectrometers generally acquire
data about a single point or line at a time, and optical
or mechanical means are used to develop a two-
dimensional image of the sample. In many cases, a fully-
25 populated image cube is obtained, consisting of an image
of the sample at each adjacent spectral band in the

spectral region of interest. With interferometers, linear-variable filters and most spectrometers, there is no way to avoid acquiring the full image cube, even if only a portion of the cube is desired. Data requirements are enormous: for high-resolution images (2048 x 3072 pixels, 12 bits/pixel) with 32 spectral bands, nearly 400 megabytes of data must be acquired and processed. Filter wheels enable selective acquisition of the wavelengths of interest, so a sparsely populated cube may be obtained. However, if there are N bands of interest, a minimum of N exposures is still required. This is quite time-consuming and inefficient.

Spectral measures have been developed that utilize many spectral bands to describe a sample attribute, or to classify regions within a sample. An example is described in "Biomedical Applications Of The Information-Efficient Spectral Imaging Sensor (ISIS)", Gentry, Steven M.; Levenson, Richard M. Proc. SPIE Vol. 3603, p. 129-142 (1999), which describes a technique called projection pursuit that relies on a specific weighting of various spectral bands to derive an enhanced detection of sample properties. Other well-known methods such as principal component analysis involve generating spectrally-weighted sums with precise control over the weighting function.

Gentry also describes an imaging system which he terms an information-efficient spectral imaging system (ISIS). ISIS provides means for imaging a line of the sample at a time through a dispersive element, followed
5 by an element that selectively transmits light in each spectral band according to a desired transmission amount, then a detector which determines the spectrally-weighted signal content at each pixel along the line. This produces an optically-weighted signal at the
10 detector, rather than requiring digitization of each spectral component followed by numerical integration. While efficient in that sense, which is a great advance over the prior art of interferometry or band-sequential filters, it only images a line at a time and so requires
15 several thousand exposures to produce a two-dimensional image. Stepping or scanning means must be provided as well, to sweep across the sample.

Use of lamps and mechanically-tuned gratings to produce monochromatic light, and thus to obtain spectral
20 images, is well-known in the art.

The use of red, green and blue LEDs for illumination is known in the art, both in time-sequential fashion or simultaneously. The colors produced by such systems are broad, ill-defined, and

would not be suitable for applications that involve precise quantitative spectral measures or indicia.

For example, in U.S. 5,838,451, McCarthy teaches the use of a handful of LEDs with broad spectral outputs (40 nm or more) to illuminate a sample, either singly or in combination to approximate standard functions such as the tristimulus curves. These are used to construct a colorimeter. To ensure stability in time, the temperature of the LEDs is precisely regulated. Such a system is unsuitable for spectroscopic measurements, nor can it obtain accurate images of spatial distributions within objects because the illumination patterns of the various LEDs are not spatially alike.

McCarthy teaches in U.S. 5,137,364 a system wherein two or more LEDs with different broad emission spectra are used to illuminate a sample and the resultant light is imaged by two or more photosensors, each of which has a different spectral responsivity. The source LEDs are turned on one at a time in sequence, and the photosensor readings are noted in each case. From the table containing readings from each photosensor under each of the illumination conditions, and a calibration, various spectral and/or colorimetric estimates of the sample are obtained.

U.S. 5,608,213 and 5,567,937 describe the use of infrared LEDs as scene simulators to test night-vision goggles with a mixture of wavelengths that approximate terrestrial scenes.

5 The use of an array of LEDs with a current switch, along with optics including a dispersive element such as a prism or grating, to produce light of time-sequential wavelength which illuminates samples for subsequent spectral analysis is described in U.S. 5,029,245 issued
10 to Keranen.

A subsequent patent, U.S. 6,075,595, uses the same apparatus as Keranen and further incorporates a substrate with embossed dimple-shaped light concentrators in which the LEDs are mounted, to restrict
15 the angles at which light emerges from the LEDs and passes into the collection optics. This is said to enhance brightness and reduce scatter.

Further discussion of LED array illuminators are contained in "Novel Spectroscopic Techniques For
20 Biomedical Applications", Hyvarinen, T.; Aikio, M.; Esko, H.; Malinen, J.; Proc. SPIE Vol. 2084, p. 224-230 (1994); and "Thirty-Two Channel Led Array Spectrometer Module With Compact Optomechanical Construction", Malinen, J.; Keranen, H.; Hannula, T.; Hyvarinen, T., in
25 "Optomechanics And Dimensional Stability; Proceedings Of

The Meeting", San Diego, CA, July 25, 26, 1991, pp. 122-128 (1991).

Ken Spring of the N.I.H. has demonstrated a system that uses three or four LEDs for time-sequential
5 excitation of fluorescent samples at distinct excitation wavelengths. The optical arrangement collimates the emission from each LED and then passes it through a bandpass filter to define a spectral band. The collimated light from the several LEDs is introduced
10 into the pupil plane of a telescope, which accepts light from whichever LED is lit, and feeds it into a multimode fiber. The fiber spatially scrambles the light and removes any inhomogeneities, after which the light emerges from the fiber and is re-imaged to illuminate
15 the fluorescent sample. This system creates light that is essentially monochromatic at any point in time.

There is nowhere disclosed in the prior art a means for imaging a two-dimensional sample so as to directly obtain an image of some spectral index at every point in
20 the sample. Nor does the prior art teach means for interactively determining such a spectral index from a first sample or region of a sample, then imaging other two-dimensional regions or samples according to that spectral index, except through the use of
25 interferometers or band-sequential filters to obtain

image cubes or the equivalent, each time an image is desired.

It is an object of the present invention to provide a system and method for obtaining images that reveal the
5 precise value of potentially complicated spectral indices at every point in a two-dimensional image, without need for acquiring a complete image cube. It is another object to provide means for determining these spectral indices in situ, along with means for
10 calibrating or compensating for the spectral response of the optics and detector used in the measurement. It is yet a further object to take advantage of spectral indices derived using techniques such as projection pursuit, principal component analysis, and the like, to
15 distinguish between species or quantify amounts of species within a sample. A further aim is to achieve these ends in an instrument of low cost and low complexity, with no moving parts. Yet another goal is that the invention can obtain RGB color images to
20 provide reference images of a sample, using the same apparatus as is employed for imaging spectral indicia.

Overall, it is the goal of this invention to provide an integrated system which can provide RGB imagery; which can provide precise band-by-band
25 measurement of standard spectral indices such as

photometric and colorimetric indices; which can take
full spectral image cubes of a sample; which can
determine quantitative, potentially complicated spectral
indices from image cubes; which can perform precise
5 calibration; and which can then rapidly image a sample
using the spectral indices of interest.

SUMMARY OF THE INVENTION

The invention comprises using the light from a spectral illuminator to illuminate a sample whose detailed spectral properties are sought. The spectral illuminator can provide light having any desired distribution of wavelengths across a broad range. It provides for independently and electronically selecting the amount of light in each of many independent wavelength bands. All bands may be on, with precisely chosen amounts of light in each band. Or, only a few bands may on at a given time, or only a single band. Near-IR versions are possible as well.

By taking a sequence of images as the illuminator is controlled to provide monochromatic light in one wavelength band after another, a spectral image cube may be obtained. Optimum signal to noise may be obtained, since the amount of light used in each exposure can be tailored via the illuminator to make use of the full dynamic range of the detector, despite spectrally varying response in the detector and other optical elements.

The invention further has as its aim to provide for directly taking images of a spectral weighted index in a sample, without need for taking an image in each individual band then numerically weighting and summing.

The spectral illuminator can be used to provide an illuminant that has precisely the spectral index as its illumination spectrum, at which point a picture may be taken that immediately records the weighted index of interest.

Since the illuminator can provide pure spectral bands as well as complex weighted spectral distributions, it provides the means for optimizing and calibrating the measurements and systems described above. It is known that detectors have a spectral response which must be accommodated in order to acquire a spectral index from a sample; the detector response can be measured by a series of monochromatic measurements using the spectral illuminator. So the illuminator may be used to correct for spectral artifacts in the rest of the optical system where its use is envisioned. In addition, the detector may be used to correct for nonlinearities, if any, in the illuminator. The photodiode sensors used in modern CMOS, CCD, and CID detectors are linear over a flux range spanning 5 orders of magnitude. To the extent that the illuminator's intensity response in a given band is nonlinear, it may be measured and corrected via the detector. When all bands are linear, and the spectral response of the system (detector plus optics) is known, then it is

possible to produce precisely calibrated illumination spectra at will.

Finally, in many cases one does not know the desired spectral weighting function a priori and it must
5 be determined. The illuminator can be used to collect an image cube of a reference or test sample, from which all spectral bands are collected. The data from this image cube is analyzed to determine the spectral weighting function, by means of principal component analysis,
10 projection pursuit, matched filtration, or any chosen method. This weighting function is then programmed into the spectral illuminator, so images using this weighting function may then be directly collected from subsequent samples. The same illuminator may be used for
15 determining the weighting function, and for obtaining the images of the samples using this spectral weighting index.

The various features of novelty which characterize the invention are pointed out with particularity in the
20 claims annexed to and forming a part of the disclosure. For a better understanding of the invention, its operating advantages, and specific objects attained by its use, reference should be had to the drawing and descriptive matter in which there are illustrated and
25 described preferred embodiments of the invention.

DETAILED DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagrammatic representation of a first implementation of a spectral illuminator 1 suitable for use in the present invention. LED lamps 10a-j produce light of various wavelengths that span the spectral range of interest (normally the photopic visible). Lenses 11a-j collimate the light from each LED and bandpass filters 12a-j provide spectral filtration of the various bands so that the light in each band has a very well-defined spectral range with much greater specificity than that defined by the LEDs themselves. Objective 13 re-images the collimated light from the various LED channels onto the tip of optical fiber 14. When light emerges from this fiber, it is imaged by lens system 15 and directed towards the sample without spectro-spatial artifacts;

Figure 2 diagrammatically illustrates another implementation of a spectral illuminator 1 for use with the present invention, wherein LED lamps 10a-j are placed at the focal plane of a spectrometer comprising reflective grating 31, lens system 32, and optional exit slit 33. Light emerging from the LEDs is spectrally selected by the grating according to the grating equation and the position of the LEDs, so that only a

precisely regulated band enters multimode optical fiber 14 and passes to the sample through lens system 15;

Figure 3 shows a block diagram of the electronics 40 used in a spectral illuminator in accordance with the invention, with control module 42, analog electronics 43a-j producing signals 44a-j for driving the LEDs, an optional memory module 41 for storing information about several drive states, and digital interface 45;

Figure 4a shows partly in block diagram form and partly diagrammatically a system 60 for obtaining spectral measurements of a sample 67 on a microscope 65 using the spectral illuminator 1 and associated control electronics 40, along with a video or digital camera 61, digitizer 62, computer 63 and software 64; and

Figure 4b shows partly in block diagram form and partly diagrammatically a system 60' for obtaining spectral measurements of non-microscopic sample 67 using the spectral illuminator 1 and its control electronics 40, along with a video or digital camera 61, lens 66, digitizer 62, computer 63 and software 64.

DETAILED DESCRIPTION OF THE PRESENTLY PREFERREDEMBODIMENTS

The preferred embodiment of the spectral illuminator uses LEDs with one LED per spectral channel.

5 The light levels can be independently controlled by varying the drive currents, since the optical output of LEDs varies linearly with current, for low currents and low frequencies. It is vital to couple the light from all LEDs into a single beam, without spectral variation

10 from point to point.

A first preferred embodiment of a spectral illuminator in accordance with the present invention is illustrated in Figure 1. It depicts a system with ten spectral channels, but this is meant to be illustrative

15 rather than restrictive, and systems may be built with any number of channels utilizing the same principles. The beam from each LED 10a-10j is collimated by lenses 11a-11j to produce a pupil plane at infinity, oriented parallel to a common optical axis. The light from the

20 lenses then passes through bandpass filters 12a-12j to provide well-defined spectral outputs. The various LEDs, collimators and filters are tiled at the entrance aperture of a telescope 13, which images all rays of a shared propagation direction to a common point. An

25 optical fiber 14 is placed at that point. The telescope

causes the spatially distinct but parallel rays from the various LEDs to pass through a single point with various angles. The optical fiber randomizes the angular distribution, provided that the length of the fiber is
5 long enough such that all rays undergo multiple reflections within it.

While a range of configurations are possible, most commonly the spectral channels will be in the range of 3 nm - 20 nm per band, and there will be from 8 to 80
10 spectral bands overall. LEDs are not presently available with spectral widths narrower than about 35 nm, so it is normally necessary to use bandpass filters to further define the passband from each given LED. For example, in Figure 1, there are 10 LEDs and bandpass filters with
15 center wavelengths spanning the range 430 nm - 655 nm at 25 nm intervals. Since the light is collimated at the point it passes through the bandpass filters, the spectral properties of those elements are not compromised.

20 The combination of each collimator 11a-11j and the telescope 13 comprises a magnifier with gain $F2/F1$, where $F2$ is the focal length of the telescope and $F1$ is the focal length of the collimator lens. The design of this assembly presents optical engineering problems as
25 will be known to one skilled in the art of optical

design. Primary goals are to produce a high efficiency in coupling light from the LED into the fiber, and to ensure the design is manufacturable without requiring unduly tight dimensional tolerances.

5 Use of a large diameter optical fiber is often favored since this enables capturing more light. Similarly, use of optical fiber with relatively high numerical aperture (NA) is favored in many designs, since the fiber NA limits the effective NA of the
10 telescope. Higher NA means a shorter telescope may be employed, yielding a more compact assembly.

Choice of particular LEDs is dictated by the need for spectral coverage and high brightness. The latter is important since the overall brightness of the
15 illuminator is proportional to the spectral radiance of the LED. It is possible to use bare LEDs of the type that emit vertically (normal to the chip surface), as well as to use packaged LEDs. Choice of one over the other will be dictated by the properties of available
20 LED sources, as well as cost, ease of assembly, and other practical factors. Suitable LEDs include high-brightness LEDs from Stanley, Cree Research, Nichia, and KingBright. These are sometimes hyperbolically described as "ultrabright", "superbright", and the like. Cree
25 provides LEDs at 430, 450, 470, 490, 505, and 525 nm,

which between them cover the range 420 - 540 nm. Many manufacturers provide LEDs covering the range 525 - 680 nm and the near-IR range, as is well-known in the art, and one source of such LEDs is MarkTech Optoelectronics (Menands, NY). When packaged LEDs can be used, the rays leaving the LED are refracted somewhat by the package, and it may be helpful in some cases to polish the LED front to be flat instead. The collimating lenses 11a-j are designed accordingly with the goal of maximizing the energy that is coupled into the fiber.

In one realization of the embodiment depicted in Fig. 1, the lenses 11 consist of 19 individual lenses, each with 25.4 mm focal length and diameter of 12.7 mm. They are tiled in a hexagonal close-packed arrangement within an overall diameter of 63.5 mm. Nineteen LEDs having wavelengths from 420 - 690 nm arranged at 15 nm intervals are situated behind the lenses, and nineteen interference filters having corresponding passbands are placed in front of the lenses. Mechanical mounting of the lenses and filters is accomplished using the space between them, so maximum packing density is achieved. The telescope consists of a lens with 65 mm diameter and 75 mm focal length, for a working NA of 0.397. The telescope couples the collimated light from these LEDs into a 3M multimode fiber with a 1 mm core and an NA of

0.39 (ThorLabs, Newton NJ, part FT-1.0-UMT). The fiber length is 2 meters, and at the distal end an achromatic lens of 25.4 mm focal length and 22 mm diameter images the fiber tip to infinity. The output beam may be
5 directly coupled into microscopes such as the Zeiss AxioPlan.

In many cases, it is preferable to use a lens array rather than individual lenses for each LED. The lenses can be plastic or glass, as long as the optical quality
10 does not degrade the efficiency of optical coupling into the fiber. Mirrors may be used instead of lenses at each and every instance, and the choice of one over the other will be dictated by cost, optical performance, and other engineering design factors known to those skilled in the
15 art.

It is not essential in the design of Figure 1 that all LEDs have the same size lens. Varying the numerical aperture (NA) of the LED collimation lens provides a means for adjusting the collection efficiency, and hence
20 for tailoring the overall output of the system. Since different LEDs have different spectral radiance properties, this can be desirable. Adjustment of LED drive current is another means for balancing or tailoring the relative output powers of the various
25 channels when each is set to its full-scale intensity

setting, i.e., different LEDs can have different full scale intensity settings determined by their respective maximum LED drive currents.

The approach in US 5,029,545 is another preferred embodiment of a spectral illuminator suitable for use in the present invention, and although it typically requires one to obtain the LEDs in chip form (which is inconvenient since they are normally supplied in packaged form), the arrangement is preferable because it has a higher theoretical optical efficiency and hence greater light output. Such a system is shown in Figure 3.

It is possible to use other elements to realize the spectral illuminator, in concert with, or instead of, the optical components described above. Indeed, any spectral illuminator is suitable, so long as it provides a plurality of well-defined bands with minimal overlap, that span the spectral ranges of interest. The flux from each band must be adjustable and reasonably reproducible, and it must be possible to have multiple bands active at one time.

When any of the above arrangements are employed to realize the spectral illuminator, it is essential to scramble the resultant beam so that its output spatial pattern is free of spectral variations to the greatest

extent possible. Otherwise, the illumination pattern will vary depending on the wavelengths used (i.e. on which LEDs are lit), and when images are taken, the overall signal at the detector will vary both due to the
5 spatial patterns in the sample properties and due to spatial patterns in the illumination beam, and these patterns will be difficult or impossible to separate out later.

Suitable scrambling elements include without
10 limitation multimode optical fibers, randomized optical fiber bundles, quartz-type or liquid-type light guides, or spatial scramblers comprising a tube-like enclosure whose internal surfaces are reflective. The latter can be constructed from a glass cylinder whose inner surface
15 is silvered, or four strip-shaped first-surface mirrors arranged to form a rectangular cylinder, or a similar arrangement. Regardless of the cross-section shape, the tube length should be several times the tube width in order to adequately scramble the beam.

20 The launch optics must also be designed to provide an intensity distribution that does not vary from wavelength to wavelength. Among the acceptable approaches are to collimate or re-image the output of the scrambler using reflective optics; and to collimate
25 or re-image the scrambler output using an achromatic or

apochromatic lens system. When the scrambler output is re-imaged, one approach is to place the sample at the focus of the re-imaging optic, so all rays sharing a given propagation vector will image to the same point on the sample. Alternatively, the scrambler output can be re-imaged onto the sample using a relay lens system, although this typically produces a less uniform range of intensities across the sample. Other arrangements that are known in the optical design art are also acceptable for launch optics, so long as they produce an intensity distribution that does not vary significantly with wavelength band.

In any of the previous embodiments, it is possible to include plural light sources for a given spectral band if higher brightness is needed than is available from a single source. Two or more sources then contribute additively to the overall flux. For example, if two or more LED elements 10a-j and filter elements 12a-j in Figure 1 address the same band, a greater flux is available. Note that in the grating-based system, the second LED must be displaced in the dimension transverse to the dispersion axis, so the flux it contributes is selected to have the proper wavelength.

It is possible to incorporate laser diode sources as well as LED sources in the spectral illuminator,

using fiberized or nonfiberized laser sources. The illuminator can thereby include one or more spectral line laser sources as well as broad-band LED sources. Means for incorporating such sources include without
5 limitation collimating the laser light and making it incident on the telescope along with the light from the LEDs; use of fiber couplers to join the laser with light from the LEDs; placing the laser amongst an array of LEDs at the appropriate location in the focal plane of a
10 spectrometer; and use of dichroic elements to join the laser light with the collimated light from one or more LEDs coupled by any means whatsoever.

It is sometimes beneficial to incorporate a laser diode to provide illumination levels, or wavelengths, or
15 modulation speeds that exceed the capability of available LEDs. At present, for example, Nichia sells a 400 nm laser diode which can be used to excite the Hoechst nuclear stain (of interest to microscopy users), but there is no source of 400 nm LEDs with comparable
20 brightness. Of course, the pace of technical development is rapid in this area, with new LED and laser diode sources becoming available, each with power and wavelength capabilities that must be matched against the application at hand. The decision to incorporate laser
25 diode elements along with the LEDs will be made

according to the required flux levels, cost of the various components, and the needs of the intended application.

Similarly, it is possible to include non-diode
5 laser sources, or even other lamp sources along with LED sources. Fibers can be used to bring the beam to a suitable place in the optics for combination with LED sources or with other laser sources. However, non-diode lasers do not normally have ready means for intensity
10 control unless the source provides modulation means internal to itself. Incorporation of shutters, variable attenuators, and other beam control means is known in the art and will be appropriate when the application warrants their use.

15 As is well-known, the output flux from an LED varies with drive current, which provides a means for precisely selecting the output of each channel. Typically a DAC converter is used together with a current-output drive circuit, with one such circuit for
20 each LED. Unused channels may be turned off entirely. The output of the array can be further time-modulated, to provide a blanking interval that may be useful in reading out digital cameras without need for a mechanical shutter, and for other purposes such as time-
25 resolved fluorescence measurements. In the latter case,

modulation circuitry may be used alone or combined with multiplying DACs to achieve higher modulation speeds. The circuitry used for this is well-known by those skilled in the art of electronic circuit design, and by
5 those familiar with circuitry used for driving LEDs for communications and photonics applications.

The overall scheme of the electronics 40 is illustrated in Figure 3, which shows a digital control module 42 that communicates with external control means
10 via an interface 45 and generates signals 44a-44j by means of circuits 43a-43j. Optional digital storage element 45 contains drive information for one or more spectral weighting functions.

When the spectral illuminator is used in
15 conjunction with a detector of some kind which has linear response, it is possible to use the detector to calibrate the illuminator. This makes use of the linear detector response to generate an in-situ calibration of the possibly nonlinear illuminator.

20 The detector is an imaging detector, meaning that the detector records the intensity at all locations across a two-dimensional grid of points. Such detectors include without limitation charge-coupled device (CCD) detectors, complementary metal-oxide semiconductor
25 (CMOS) detectors, charge-injection device (CID)

detectors, vidicon detectors, reticon detectors, image-intensifier tube detectors, and pixelated photomultiplier tube (PMT) detectors. Some of these detectors have useful features, such as being able to
5 read out only a portion of the image area when this is desired, or providing adjustable spatial resolution by means of binning several pixels together. These are consistent with the invention, and may be incorporated if this is deemed beneficial. Any detector which is an
10 imaging type and has suitable properties such as spatial resolution, sensitivity, and signal-to-noise can be employed, and the choice of one detector over another will be made for the usual engineering reasons such as cost, size, quality, readout speed, and so on.

15 A system in accordance with the invention for making spectral image measurements with a microscope is illustrated in Figure 4a. The spectral illuminator 1 is the embodiment of Figure 2 and uses a grating to combine outputs from an array of LEDs. This light is spatially
20 scrambled by a multimode optical fiber and the output from the fiber is then collimated by an achromatic lens, through which it passes to the transmitted light lamp port of a Zeiss AxioSkop (Thornwood, NY). A Roper Scientific CoolSnap camera (Tucson, AZ) 61 is attached
25 to the camera port, and is connected to a PC/pentium

computer 63 that is also connected to the interface of the spectral illuminator. Software controls the illuminator and takes images from the camera.

In one preferred embodiment, the software first
5 operates the illuminator to produce red, then green, then blue illumination, taking an image at each setting with the digital camera and merging them into a high resolution color image that is presented on the computer screen. This embodiment is a pathology workstation, and
10 the images are of tissue samples. The RGB image is used in order to read the sample and identify features of interest. From the RGB image a pathologist selects certain cells which are believed to be cancerous or otherwise of interest, by selecting them or the region
15 including them. The software then takes spectral cubes by programming the spectral illuminator to produce monochromatic light of a first wavelength, then a second, and so on, through the entire spectral range, while acquiring images at each spectral setting. The
20 digital camera may be set to acquire only the region of interest (ROI) to the pathologist, so data acquisition is quite rapid. Typically, the ROI can be 1/10 of the image or less in either width or height, so only 1 percent or less of the image area. It is possible to

acquire at nearly video rates, and the image cube of the ROI is often acquired in under two seconds.

From the spectral cube, a spectral weighting index is derived by projection pursuit, or by any other spectral algorithm of interest. These are described in the prior art such as "ISIS; An Information-Efficient Spectral Imaging System", by W. C. Sweatt, C. A. Boye, S. M. Gentry, M. R. Descour, B. R. Stallard, C. L. Grotbeck, *Imaging Spectrometry IV*, Proc. SPIE, Vol. 3438, pp. 98-106, San Diego, 1998; "Construction Of Filter Vectors For The Information-Efficient Spectral Imaging Sensor", by B. R. Stallard, S. M. Gentry, *Imaging Spectrometry IV*, Proc. SPIE, Vol. 3438, pp. 172-182, San Diego, 1998; "Spectrally Adaptive Light Filtering", by P.I. Shnitser, I.P. Agurok, Proc. SPIE, vol. 3140, p.117-27, 1997; "High Dimensional Feature Reduction via Projection Pursuit", by L. O. Jimenez, D. Landgrebe, TR-ECE 96-5, School of Electrical Engineering, Purdue University, West Lafayette, IN 47907-1285, 1995; all of whose contents are incorporated by reference in their entirety.

Principal component analysis is another way to generate spectral distributions that are of interest for a given type of scene. Convex-hull algorithms also generate spectral distributions of interest: this method

seeks to identify what the spectra of various pure species would be, even if the species are always intermixed in the scene.

Once a spectral weighting index is derived, the spectral illuminator is then set to produce the spectral distribution specified by the spectral weighting index, and a two dimensional image of the sample is acquired. If several spectral measures are desired, then several exposures are taken. However, only one exposure is required for each spectral measure. These weighted images are analyzed for correlation with the spectral index, and cells throughout the image that are similar to the cells identified by the pathologist are identified. This identification can be performed by simply displaying the images obtained with the spectral weighting function (or by use of a false-color index for one image or by using a ratio or balance of several images taken with different weighted spectral indicia); or, it can be performed by highlighting the cells within an RGB display that have the trait of interest, so they appear as highlighted within an otherwise familiar lifelike image.

A similar workstation can be produced that is a general purpose bio-medical workstation capable of analyzing samples of blood and other vital fluids. In

such a workstation, the invention is capable of providing RGB reference images; of taking full image cubes of the entire sample or of a representative portion; of developing spectral indices based on the data taken from the representative portion; then of rapidly acquiring images of the spectral indicia over a larger sample or over many samples. The ability to take and analyze spectral image cubes of representative samples enables one to overcome many of the problems that traditionally confound spectral imaging of complex biological samples, namely those problems related to the sample preparation, staining protocol, and subtle variations that arise due to uncontrolled factors such as pH and electrolyte levels. From the training set, the degree and nature of these variations is accommodated, and a more robust measurement or assay is obtained.

It is of great benefit that no additional apparatus is required to take the detailed spectral cube images, and that the subsequent data acquisition can proceed reliably and rapidly using the spectral measures abstracted from it, using only a very few exposures. This represents a substantial advantage over present alternatives for spectral imaging, in terms of speed, robustness, and cost.

One can show the equivalence of the image data obtained using the present invention to those obtained by prior art methods. In general, the signal induced at a detector by light in an infinitesimal spectral band
5 centered at λ_0 and having width $d\lambda$ will be given by

$$D(\lambda_0) = I(\lambda_0) S(\lambda_0) R(\lambda_0) d\lambda$$

[1]

where $D(\lambda)$ is the signal produced at the detector, $I(\lambda)$ is the illumination flux, $S(\lambda)$ is the spectral response
10 of the sample, and $R(\lambda)$ is the detector responsivity at wavelength λ .

$S(\lambda)$ may indicate reflection, transmission or scatter, depending on how the sample is being illuminated and viewed, (i.e. by a transmissive,
15 reflective, or scattering arrangement). In the present context, these are equivalent so long as the wavelength of light is substantially unaltered by its interaction with the sample.

It is possible to rewrite the above equation in
20 terms of discrete bands, provided that the bands are chosen to be sufficiently narrow that neither the illumination, sample response, or detector responsivity varies greatly within a given band. Then one may write

the expression for the contribution from light in the j -th band as

$$D(j) = I(j) S(j) R(j) d\lambda_j$$

[2]

5 One normalizes spectral readings against a standard such as a white card (for reflection measurements) or a transparent reference (for transmission measurements). This normalization is performed on a band-by-band basis, and typically for the standard $S(\lambda) = 1$, leading to a
10 signal of

$$D(j) = I(j) R(j) d\lambda_j$$

[3]

when the standard is read. This allows one to measure the normalized sample property $S(j)$ by taking the ratio
15 of the detector readings when the sample is present, relative to when the standard is present, as:

$$S(j)_{\text{measured}} = D(j)_{\text{sample}} / D(j)_{\text{standard}} = I(j) S(j) R(j) d\lambda_j / I(j) R(j) d\lambda_j = S(j) \quad [4]$$

The present invention uses this same method to
20 determine the image cube for a sample. One illuminates with each wavelength band in turn and observes the detector reading $D(j)$, under both sample and standard conditions; the ratio yields the sample response $S(j)$.

But the readings $D(j)_{\text{standard}}$ are especially
25 significant for the present system. They indicate the

reading for a given spectral band at the LED current setting used for that exposure. Since the LED output is variable, it is possible to adjust the LED current setting so as to attain a sought-for reading at the
5 detector, which we denote as K . By doing so in all bands, and recording the drive current settings $C(j)$ required to meet this condition, one can calculate a calibration table of scale factors $\alpha(j)$:

$$\alpha(j) = K / C(j)$$

10 [5]

The scale factor $\alpha(j)$ is the detector response per unit of LED drive current in band j , and it will be used in realizing the spectral weighting functions, as described below.

15 The spectral weighting function WF and its distribution $F(j)$ are derived from the image cube in an entirely conventional manner, using all the methods that are familiar to one skilled in the art of spectroscopy and multispectral image analysis. Given a spectral
20 weighting function with a spectral distribution $F(j)$ for a specified set of bands λ_j , the spectral index for a sample with response $S(j)$ is given by:

$$WF = \sum_{all j} [S(j) F(j) d\lambda_j]$$

[6]

25 where $\sum_{all j}$ denotes the summation over all bands j .

Prior-art methods obtain this by first measuring the detector response $D(j)$ under standard conditions, then, when the sample is present, dividing to obtain the sample spectrum $S(j)$, and finally weighting the readings
 5 $S(j)$ by the various factors $F(j)$ and numerically integrating in accordance with Equation 5.

In contrast, the present invention adjusts the settings of the spectral illuminator to produce an illumination spectrum with the property that

$$10 \quad I(j) = F(j) / D(j)$$

[7]

That is, the illumination distribution has the shape of the spectral weighting distribution $F(j)$, divided by the detector responsivity $D(j)$.

15 When the sample is imaged under this controlled illumination, the total signal at the detector D is given by

$$\begin{aligned} D &= \sum_{all j} [I(j) S(j) D(j) d\lambda_j] \\ &= \sum_{all j} [F(j)/D(j) * S(j) * D(j) d\lambda_j] \\ 20 \quad &= \sum_{all j} [S(j) F(j) d\lambda_j] = WF. \end{aligned}$$

[8]

The detector directly measures the weighting function, without need for breaking the light up into its constituent spectrum and numerically analyzing it. The
 25 result is an optical, rather than numerical, evaluation

of the spectral weighting function, with enormous increase in speed and efficiency.

The LED current settings which realize the required illumination spectral distribution can be calculated
5 directly from the tabulated $\alpha(j)$ values; the proper LED current setting for band j is simply $F(j) / \alpha(j)$.

The importance of this result cannot be overstated. It renders spectral imaging practical for a wide range of uses where the presently-available alternatives are
10 rejected by all but the research community as far too cumbersome. By substituting a direct optical measurement for an extensive numerical computation, i.e. by replacing the acquisition and processing of an entire image cube (which may require extensive calculations on
15 128 megabytes of data or more) with the acquisition of a very few precisely weighted spectral images that directly capture the information of interest, the traditional barriers to multispectral imaging are completely removed.

20 For the first time, the process of multispectral imaging can proceed in real-time, eliminating the normal delay of several seconds per image (or the alternative need for specialized, expensive image processing hardware). The need for vast amounts of computer memory,
25 are eliminated, since typically only 2-5 bands are

required - scarcely more than is used in a normal display. A high degree of flexibility is gained, since the same apparatus can be used to perform the spectral calibration, obtain the informational RGB images, determine the spectral index of interest, and acquire the weighted images at high speed using the index and calibration performed earlier.

Another embodiment is shown in Figure 4b. Here light from a spectral illuminator is fed through illumination optics to produce a spectrally uniform illumination pattern at a macroscopic sample which is then imaged with a lens by a camera. The camera's digitized output is fed to a computer which produces images and digitized data of the sample.

The system can be used for hyperspectral imaging, or for producing an RGB image of the sample, or for imaging the sample in XYZ colorimetric space, from which representations such as L^*a^*b and Luv may be derived without loss or approximation.

In these cases, it is important to understand that the tristimulus curves are not themselves the weighting function of interest. Recall that the goal is to measure the amount of energy that a detector (like the eye) having tristimulus response would see when the sample is illuminated with a standard illuminant such as CIE A, or

daylight, or some other reference illuminant of interest. The spectral weighting function is thus the band-by-band product of a reference illumination spectrum, times one of the tristimulus functions. The
 5 result is that one obtains images of a scene as it would appear under various controlled illuminants, in accurate colorimetric coordinates.

In detail, the desired weighting function $F(j)$ is the product of the

$$10 \quad F(j) = L(j) * X(j) \quad \{X \quad \text{tristimulus reading}\} \quad [9a]$$

$$F(j) = L(j) * Y(j) \quad \{Y \quad \text{tristimulus reading}\} \quad [9b]$$

$$15 \quad F(j) = L(j) * Z(j) \quad \{Z \quad \text{tristimulus reading}\} \quad [9c]$$

where $L(j)$ is the spectrum of the desired illuminant in band (j) , and $X(j)$, $Y(j)$, and $Z(j)$ are the usual tristimulus functions, evaluated in band (j) . And to realize this measurement, one sets the spectral
 20 illuminator so that the bands are further corrected for the detector's spectral response $D(j)$, as discussed above:

$$I(j) = L(j) * X(j) / \alpha(j) \quad \{X \quad \text{tristimulus reading}\} \quad [10a]$$

$$I(j) = L(j) * Y(j) / \alpha(j) \quad \{Y$$

tristimulus reading} [10b]

$$I(j) = L(j) * Z(j) / \alpha(j) \quad \{Z$$

tristimulus reading} [10c]

5 The image thus obtained will then be the desired X, Y or
Z value as would be expressed under the specified
standard illumination such as CIE A, daylight, and so
on.

Prior-art systems having only a few LEDs with broad
10 spectral response have been designed to match the X, Y
or Z functions without regard for the illumination
conditions under which they are evaluated. Effectively,
this means the colorimetric values were obtained under a
"greybody" illuminant, which is not physically
15 appropriate for most cases, and is not normally desired.

The present system, with precise and independent
spectral control over each band, can incorporate much
more subtle effects in its action. It can readily
incorporate the effect of a suitable illumination
20 function, or of various illuminants as desired, to
assess the colorimetric values under a chosen
illumination.

The same system can be used to image the sample in
terms of spectral weighting functions that are indicia
25 of a state or condition that is peculiar to the type of

sample being imaged, and are determined by a representative measurement of the sample. As discussed in the previous embodiment, the process for doing so involves the generation of an image cube, development of
5 a weighting function $F()$, calculation of the LED current settings that realize the desired controlled illumination state $I(j)$, followed by direct imaging of the weighted spectral index using that controlled illumination. The different modes are produced by
10 software selection of the spectral illumination function $I(j)$ produced by the spectral illuminator.

A macro imaging system has utility in endoscopic applications such as surgery, for several reasons. First, the use of a sequential illumination system with
15 a monochrome camera produces sharper images than conventional RGB cameras, since the latter use a mosaic of color spots on the detector that degrade its spatial resolution by a factor of 4 compared to a monochrome detector of the same size. So, sharper pictures are
20 obtained for a given endoscope detector size, or alternatively a smaller detector can be used. Then, the surgeon may use the spectral weighting functions to identify the spectrum of a feature of interest (such as a lesion or a cancerous piece of tissue), by taking an
25 image cube of the region of interest. This is

considerably faster than for competitive techniques, since the region is normally only a small portion of the image, and the spectral illuminator can change its output in microseconds. When the spectral signature of the feature is identified (by principal component analysis, projection pursuit, or other algorithms that are known in the art), the spectral illuminator can rapidly and efficiently visualize the scene and continue hunting for other areas that have the desired spectral properties. The task of searching for low-probability events is practical since each image requires only a few exposures, one with the illuminator set to each of the spectral weighting functions of interest.

Another application of this invention is in a retinal imaging station. The spectral illuminator and associated launch optics are used to generate illumination that is spectrally agile and essentially free from variations in spatial pattern between spectral bands. A camera such as a fundus camera is used to image the retina. RGB images can be obtained by sequentially illuminating with the three color primaries while taking images with a monochrome detector. Spectral image cubes may be obtained, and the system calibrated, by sequential illumination of the various spectral bands while imaging with the monochrome imaging detector. It

is also possible to capture images of weighted spectral indicia in rapid time sequence, while imaging with the monochrome detector.

In this example, the multiple operating modes
5 provide powerful versatility. The invention enables one to obtain reference images, images of indicia of interest which can be overlaid in false color on top of the reference RGB images for display purposes, and full image cubes for the most detailed analysis. There is no
10 need for additional equipment, which eliminates bulk, expense, and complexity. The ability to take all these images at video frame rates, and in the case of the weighted indicia, to obtain vast information in only a few frames, minimizes the problem of eye movement and/or
15 the need to register various image bands later in software. From the perspective of the clinician and patient alike, the simplicity and speed that accrue from this invention are a significant benefit over present alternatives such as interferometric or laser-based
20 ophthalmic imaging systems.

It can often be useful to incorporate bands which are spectrally distinct from the visible range to improve the specificity of the spectral index. That is, adding other spectral bands to the visible bands may
25 enhance the ability to discriminate between different

types of samples, or to quantify the properties of a sample. These bands are included in the image cube, and analyzed when determining the spectral index. Although they are spectrally distinct, there is no modification
5 to the method or mathematics that is employed. If the band is useful in classifying the sample regions, it is included in the spectral weighting function and that element is turned on proportionally to its presence in that function for each exposure.

10 This is readily achieved in the near-infrared range, where LEDs and laser diodes are available in bands near 730 nm , 780 nm, 830 nm, 940 nm, and others. The choice to include one or more such bands is application-specific. For example, in the endoscopy
15 embodiment, it may be beneficial to include wavelength bands at which tissue reflectivity tends to vary strongly according to the amount and oxygenation of hemoglobin, as well as adjacent bands. There is a great deal of prior art on the use of near-infrared
20 spectroscopy to quantify total hemoglobin, blood oxygenation, blood sugar levels, and the like. This art teaches specific spectral bands in the near-infrared for this purpose. These bands may be included with visible illuminants, to enhance the selectivity of the spectral
25 weighting functions that discriminate between different

sample states or properties. The spectral illuminator is then used to realize such weighting functions and directly image the sample properties in these terms.

The use of medical examples is not meant to suggest
5 that this is the sole or predominant use of the present invention. Similar benefits accrue in the use of borescopes for industrial inspection, in reflected-light microscopy for semiconductor and materials measurements, and in any endeavor where spectral signatures enable
10 discrimination between sample regions or quantify sample properties. One such application is in the field of counterfeit detection, since in general the dyes, paints, and materials used in a counterfeit will not match the genuine article at all wavelengths even if the
15 overall visual appearance is quite similar. The phenomenon whereby objects appear similar to the eye under one set of lighting, but appear quite different under another set of lighting, is termed metamerism and can be used to great advantage in this context. A
20 spectral weighting function that maximally illuminates in the spectral regions where the genuine article and the fake are different, will highlight these distinctions and make them readily apparent. The present invention provides a means for at once identifying the
25 weighting function (through taking of a spectral image

cube and its subsequent analysis on a computer), and then providing essentially real-time imaging of the possible counterfeit objects using that derived spectral weighting function (by having the spectral illuminator
5 provide the weighted illuminants while imaging the sample).

It is also possible to alternate the spectral illuminator between one spectral weighting function and another, at a rate of a few cycles per second, so that
10 one can view the object directly (or in the eyepieces of the microscope) in a way that will highlight the objects of interest by maximizing the visual contrast between settings for objects with the desired spectral distribution. Ideally, the spectral weighting function
15 should take account of the disparity between the detector that was used to determine the optimal spectral weighting function, and the spectral sensitivity of the human eye, and adjust the settings of each spectral band in the spectral illuminator accordingly.

20 Except as specifically noted, a range of specific designs, components and optical elements may be used to realize the invention, and such alternatives and equivalents may be employed without deviating from the spirit of the invention, as will be apparent to one

skilled in the field of optical design and instrument design.

It should also be understood that any reference in the claims or the specification to a "means for" accomplishing a function of an apparatus or a step in a process is intended to encompass any implementation thereof known to those of skill in the art and that no limitation whatsoever on the manner of said implementation is intended, except insofar as necessary to achieve the intended results of the invention. For example, any reference in the claims to a computer having "means for" accomplishing a function is intended to encompass any software or hardware implementation capable of accomplishing that function.

Thus various embodiments and components have been shown for constructing this invention, and these may be used singly or in combination, or with other elements known in the arts of optical design, color science, and instrument design. It is understood that these and other such combinations, substitutions, and alternative embodiments may be undertaken according to the requirements and materials at hand without deviating from the spirit of the invention, the scope of which is to be limited only by the claims appended hereto.

CLAIMS

We claim:

1. An imaging spectroscopy system, comprising:
 - a spectral illuminator that emits light in a plurality of spectral bands within a predefined spectral range, with the intensity of light in each spectral band being independently adjustable;
 - drive circuitry that controls the flux in each spectral band of the spectral illuminator;
 - 10 scrambling optics that spatially homogenize the distribution of light emitted by the spectral illuminator;
 - beam delivery optics that direct the spatially homogenized light from the scrambling optics to form an illumination pattern at a sample, wherein the illumination pattern is substantially free of variation as a function of wavelength within the predefined spectral range;
 - receiver optics that receive light which has
20 interacted with the sample and form an image of the sample at a focal plane;
 - an imaging detector located at the focal plane for detecting an image of the sample; and
 - a computer in communication with the drive
25 circuitry and the imaging detector for controlling the

drive circuitry and receiving data from the imaging detector.

2. The imaging spectroscopy system of claim 1 wherein
5 the spectral illuminator emits light in plural bands simultaneously, with different intensity levels in each active band.
3. The imaging spectroscopy system of claim 2 wherein
10 the spectral illuminator emits light with precisely controlled ratios of intensity between plural spectral bands.
4. The imaging spectroscopy system of claim 1 wherein
15 the spectral illuminator comprises an array of LEDs coupled to an output slit by means of a dispersive element.
5. The imaging system of claim 1 wherein the receiver
20 optics are not spectrally selective and have high transmission at all bands within the predefined spectral range.
6. A method of spectral imaging comprising:

obtaining reference images at a plurality of pure spectral bands by controlling a spectral illuminator to sequentially produce light in each of said plurality of pure spectral bands and recording an image under these
5 conditions using an imaging detector;

calibrating the system spectral response using the reference images; and

measuring the value of one or more spectral weighting functions in the sample by repeating the
10 following steps for each spectral weighting function:

i) adjusting the spectral illuminator to produce light whose spectral flux distribution is based upon the calibration data and the spectral weighting function;

15 ii) illuminating the sample with light from the spectral illuminator as adjusted in step (i);
and

(iii) recording an image of the sample under these illumination conditions using the
20 imaging detector,

wherein each of said one or more spectral weighting functions indicates a property or attribute of the sample and comprises a plurality of components in different spectral bands.

7. The method of claim 6 wherein there are plural weighting functions.

8. The method of claim 6 wherein the value of at least
5 one spectral weighting function is the product of a tristimulus function and a known illumination function.

9. The method of claim 8 wherein the known illumination function is one of the CIE A, CIE C,
10 daylight, and a 5600K blackbody illumination functions.

10. The method of claim 9 further comprising determining at least a first and a second spectral weighting functions, wherein the first spectral
15 weighting function is a tristimulus function multiplied by a first illumination function, and the second spectral weighting function is a tristimulus function multiplied by a second illumination function, and the first and second illumination functions are distinct.

20

11. A method of imaging comprising:

obtaining images of a first sample at a plurality of pure spectral bands by controlling a spectral illuminator to sequentially produce light in each of
25 said plurality of pure spectral bands and recording

images of the first sample under these conditions using an imaging detector;

determining one or more spectral weighting functions that indicate a property or attribute of a sample, using the images of the first sample, wherein
5 each spectral weighting function comprises a plurality of components in different spectral bands; and

measuring the value of one or more of said spectral weighting functions in a subsequent sample by repeating
10 the following steps for each of said one or more spectral weighting functions:

- i) adjusting the spectral illuminator to produce light whose spectral flux distribution is based upon the spectral weighting function,
- 15 ii) illuminating the sample with light from the spectral illuminator as adjusted in step (i); and
- iii) recording an image of the second sample under these illumination conditions using the
20 imaging detector.

12. The method of claim 11 wherein the spectral weighting function is determined by one of principal component analysis, projection pursuit, independent
25 component analysis, and convex-hull analysis.

13. The method of claim 11 wherein the spectral weighting function is determined by projection pursuit analysis.

5

14. The method of claim 11 wherein the spectral weighting function is determined by principal component analysis.

10 15. The method of claim 11 wherein plural spectral weighting functions are determined.

16. A method of spectral imaging comprising:

obtaining reference images at a plurality of pure
15 spectral bands by controlling a spectral illuminator to sequentially produce light in each of said plurality of pure spectral bands and recording an image under these conditions using an imaging detector;

calibrating the system spectral response using the
20 reference images;

obtaining images of a first sample at a plurality of pure spectral bands by controlling a spectral illuminator to sequentially produce light in each of said plurality of pure spectral bands and recording

images of the first sample under these conditions using an imaging detector;

determining one or more spectral weighting functions that indicate a property or attribute of a sample, using the images of the first sample, wherein
5 each spectral weighting function comprises a plurality of components in different spectral bands; and

wherein a spectral weighting function comprises a plurality of components in different spectral bands;

10 measuring the value of one or more spectral weighting functions in a subsequent sample by repeating the following steps for each of said one or more spectral weighting functions:

i) adjusting the spectral illuminator to
15 produce light whose spectral flux distribution is based upon the calibration data and the spectral weighting function, and

ii) illuminating the sample with light from the spectral illuminator as adjusted in step (i);
20 and

iii) recording an image of the second sample under these illumination conditions using the imaging detector.

17. The method of claim 16 wherein plural spectral weighting functions are determined.

18. A pathology workstation comprising:

5 a. spectral illuminator that emits light in a plurality of spectral bands within a predefined spectral range, with the intensity of light in each spectral band being independently adjustable;

 drive circuitry that controls the flux in each
10 spectral band of the spectral illuminator;

 scrambling optics that spatially homogenize the distribution of light emitted by the spectral illuminator;

 beam delivery optics that direct the spatially
15 homogenized light from the scrambling optics to form an illumination pattern at a sample, wherein the illumination pattern is substantially free of variation as a function of wavelength within the predefined spectral range;

20 receiver optics that receive light which has interacted with the sample and form an image of the sample at a focal plane;

 an imaging detector located at the focal plane for detecting an image of the sample; and

a computer in communication with the drive circuitry and the imaging detector for controlling the drive circuitry and receiving data from the imaging detector.

5

19. The pathology workstation of claim 18 wherein, for said sample, said computer acquires data from the imaging detector at each of a plurality of pure spectral bands.

10

20. The pathology workstation of claim 18, wherein, based on the data received from the imaging detector, said computer is capable of generating color images of said sample and generating an image of a spectral weighting function at every point in a two-dimensional sample field in a single exposure.

21. The pathology workstation of claim 18, wherein the computer acquires from said imaging detector data for said sample at each of a plurality of pure spectral bands and determines one or more spectral weighting functions from said data.

22. The pathology workstation of claim 21 wherein the computer can generate an image of each spectral

25

weighting function at every point in a two-dimensional sample field in a single exposure.

23. A blood analysis workstation comprising:

- 5 a spectral illuminator that emits light in a plurality of spectral bands within a predefined spectral range, with the intensity of light in each spectral band being independently adjustable;
- drive circuitry that controls the flux in each
- 10 spectral band of the spectral illuminator;
- scrambling optics that spatially homogenize the distribution of light emitted by the spectral illuminator;
- beam delivery optics that direct the spatially
- 15 homogenized light from the scrambling optics to form an illumination pattern at a sample, wherein the illumination pattern is substantially free of variation as a function of wavelength within the predefined spectral range;
- 20 receiver optics that receive light which has interacted with the sample and form an image of the sample at a focal plane;
- an imaging detector located at the focal plane for detecting an image of the sample; and

a computer in communication with the drive circuitry and the imaging detector for controlling the drive circuitry and receiving data from the imaging detector.

5

24. The blood analysis workstation of claim 23 wherein, for said sample, said computer acquires data from the imaging detector at each of a plurality of pure spectral bands.

10

25. The blood analysis workstation of claim 24 wherein the computer calibrates the overall spectral response of the imaging detector based on the data from the imaging detector at each of said plurality of pure spectral

15 bands.

26. The blood analysis workstation of claim 25 wherein the computer includes means for generating an image of a spectral weighting function at every point in a two-dimensional sample field in a single exposure.

27. The blood analysis workstation of claim 24 wherein the computer includes means for determining one of measurement fidelity and sample quality based on the

data acquired from the imaging detector at each of said plurality of pure spectral bands.

28. The blood analysis workstation of claim 23 wherein
5 the computer includes means for determining one or more spectral weighting functions based on the data acquired from the imaging detector at each of said plurality of pure spectral bands, and

wherein the computer includes means for generating
10 an image of a spectral weighting function at every point in a two-dimensional sample field in a single exposure.

29. An endoscopy imaging station comprising:

a spectral illuminator that emits light in a
15 plurality of spectral bands within a predefined spectral range, with the intensity of light in each spectral band being independently adjustable;

drive circuitry that controls the flux in each spectral band of the spectral illuminator;

20 scrambling optics that spatially homogenize the distribution of light emitted by the spectral illuminator;

beam delivery optics that direct the spatially homogenized light from the scrambling optics to form an
25 illumination pattern at a sample, wherein the

illumination pattern is substantially free of variation as a function of wavelength within the predefined spectral range;

receiver optics that receive light which has
5 interacted with the sample and form an image of the sample at a focal plane;

an imaging detector located at the focal plane for detecting an image of the sample; and

a computer in communication with the drive
10 circuitry and the imaging detector for controlling the drive circuitry and receiving data from the imaging detector.

30. The endoscopy imaging station of claim 29 further
15 comprising a display for displaying full image cubes and RGB reference images.

31. The endoscopy imaging station of claim 29 wherein
said computer includes means for determining spectral
20 indicia based on full image cubes.

32. The endoscopy imaging station of claim 29, further
comprising a display, and wherein said computer includes
means for generating on said display an RGB reference
25 image of the sample based on data from the imaging

detector and means for overlaying on said RGB reference image an image of said sample obtained by adjusting said spectral illuminator for a spectral weighting function.

5 33. A retinal imaging station comprising:

a spectral illuminator that emits light in a plurality of spectral bands within a predefined spectral range, with the intensity of light in each spectral band being independently adjustable;

10 drive circuitry that controls the flux in each spectral band of the spectral illuminator;

scrambling optics that spatially homogenize the distribution of light emitted by the spectral illuminator;

15 beam delivery optics that direct the spatially homogenized light from the scrambling optics to form an illumination pattern at a sample, wherein the illumination pattern is substantially free of variation as a function of wavelength within the predefined
20 spectral range;

receiver optics that receive light which has interacted with the sample and form an image of the sample at a focal plane;

an imaging detector located at the focal plane for
25 detecting an image of the sample; and

a computer in communication with the drive circuitry and the imaging detector for controlling the drive circuitry and receiving data from the imaging detector.

5

34. The retinal imaging station of claim 33 wherein said computer generates full image cubes for said sample based on data from said imaging detector.

10 35. The retinal imaging station of claim 34 wherein said computer includes means for generating a spectral weighting function for said sample based on said full image cubes.

15 36. A method of imaging comprising using a spectral illuminator, launch optics, imaging detector with step of designing launch optics to minimize spectro-spatial artifacts.

1 / 5

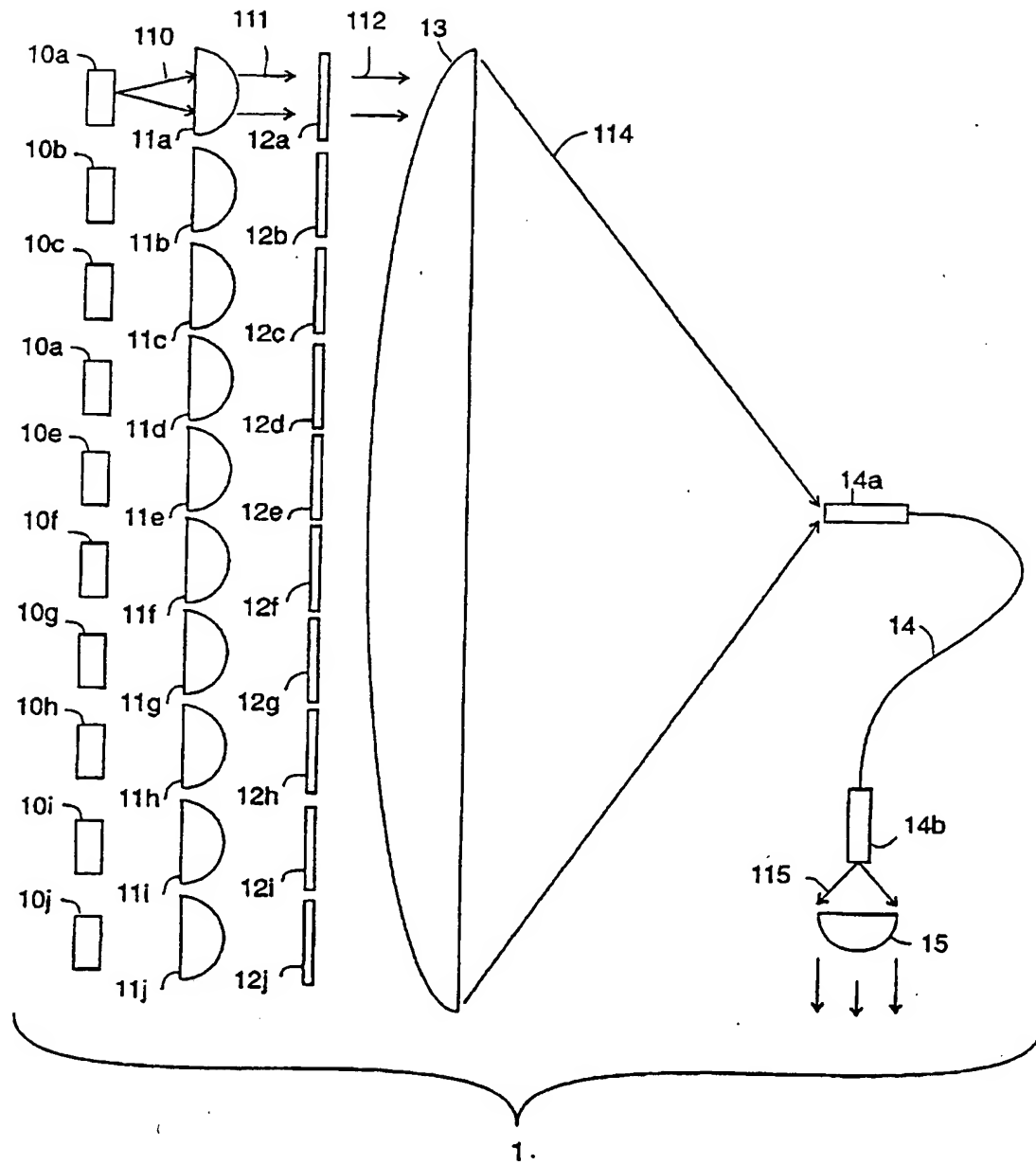


Fig. 1

2 / 5

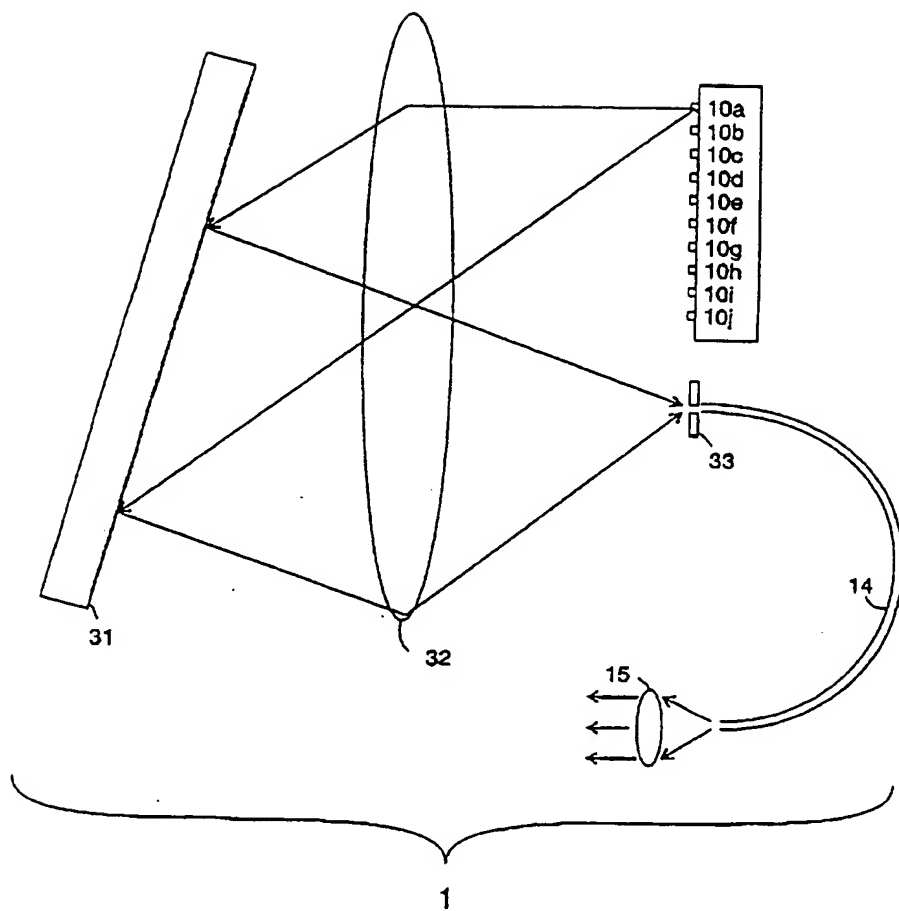


Fig. 2

3 / 5

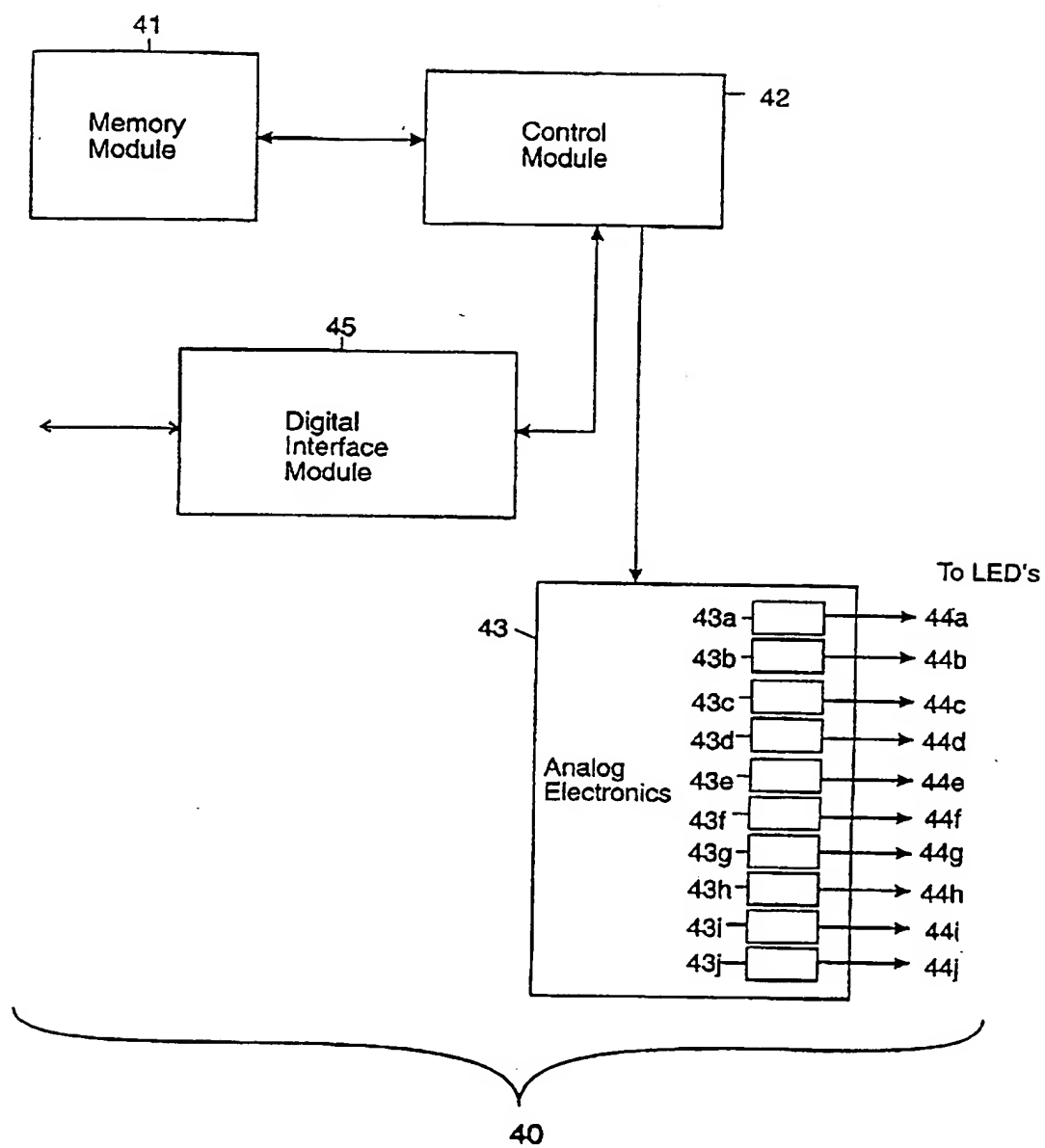


Fig. 3

4 / 5

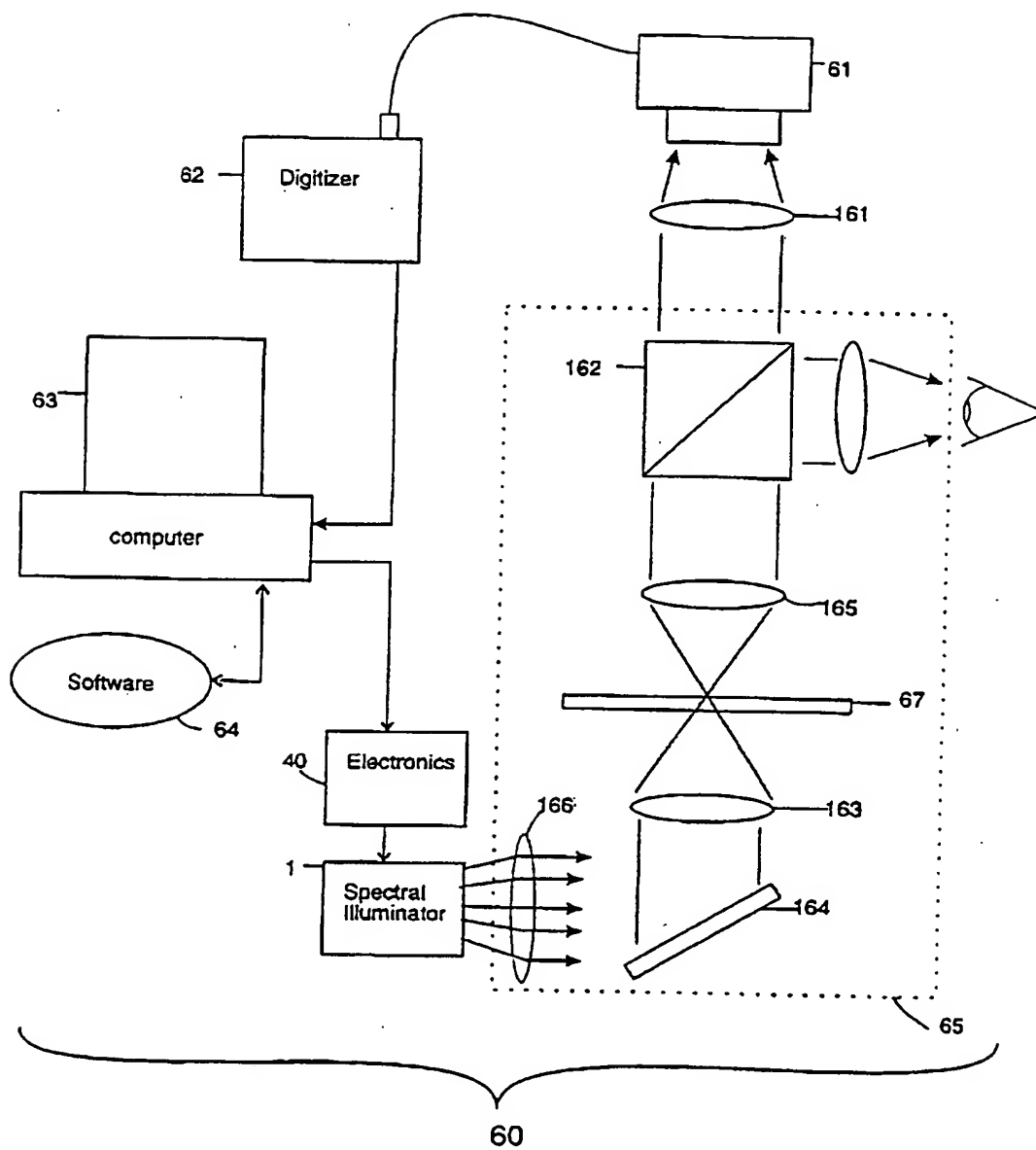


Fig. 4a

5 / 5

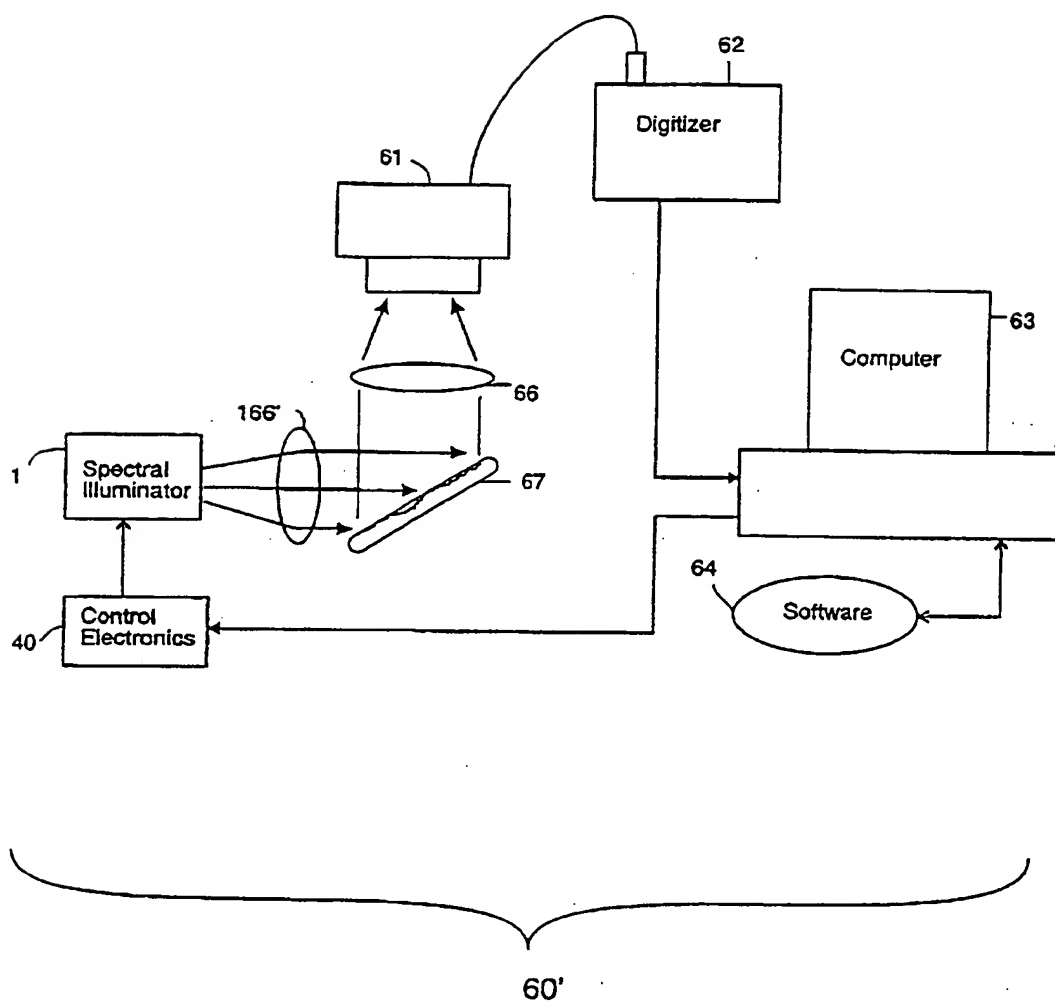


Fig. 4b

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/21481

A. CLASSIFICATION OF SUBJECT MATTER														
IPC(7) : G01N 21/64; G01J 5/02 US CL : 356/326, 318, 328; 250/339 According to International Patent Classification (IPC) or to both national classification and IPC														
B. FIELDS SEARCHED														
Minimum documentation searched (classification system followed by classification symbols) U.S. : 356/326, 318, 328; 250/339														
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched NONE														
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) USPTO APS, EAST search terms: image cube, spectrum, array, filters, optical fibers, waveguides, spectroscopy														
C. DOCUMENTS CONSIDERED TO BE RELEVANT														
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.												
Y	US 4,519,707 A (KUFFER) 23 May 1985 (23.05.1985), see entire document.	1-36												
Y	US 5,066,124 A (WULF) 19 November 1991 (19.11.1991), see col. 2, line 53 - col. 3, line 30.	1												
Y,E	US 6,160,618 A (GARNER) 12 December 2000 (12.12.2000), see entire document.	1-36												
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.														
<table border="0"> <tr> <td>* Special categories of cited documents:</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"E" earlier document published on or after the international filing date</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"&" document member of the same patent family</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td></td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family	"O" document referring to an oral disclosure, use, exhibition or other means		"P" document published prior to the international filing date but later than the priority date claimed	
* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention													
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone													
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art													
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family													
"O" document referring to an oral disclosure, use, exhibition or other means														
"P" document published prior to the international filing date but later than the priority date claimed														
Date of the actual completion of the international search 29 DECEMBER 2000		Date of mailing of the international search report 19 JAN 2001												
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer ZANDRA V. SMITH Telephone No. (703) 305-7776 <i>James P. Gustin</i>												